Expression of subunit *III* ot the ATP synthase from spinach chloroplasts in *Escherichia coli*

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Expression of subunit *III* of the ATP synthase from spinach chloroplasts in *Escherichia coli* has been achieved. Although the protein is inserted into the bacterial cytoplasmic membrane, formation of a functional F_0 complex was not observed.

F_1F_0; ATP synthase; F_0 complex; Proteolipid; Complementation

1. INTRODUCTION

ATP synthases (F_1F_0) are located in energy-converting membranes of mitochondria, chloroplasts and bacteria, where they catalyze the synthesis of ATP from ADP and inorganic phosphate using an electrochemical proton gradient across the membrane. In bacteria the enzyme can also function in the reverse direction by coupling the hydrolysis of ATP to the translocation of protons [1-4]. The ATP synthase consists of two different subcomplexes, F_1 and F_0. The water-soluble F_1 portion carries the catalytic sites for ATP synthesis and hydrolysis. It is associated with the membrane-embedded F_0 moiety which forms the proton channel.

The most extensively studied ATP synthase is that of *Escherichia coli*. It is encoded by a single operon (*unc*) located at about 83 min on the bacterial chromosome [5]. In contrast, the spinach chloroplast ATP synthase is encoded by three nuclear genes and by two operons, designated A and B, located on the plastid genome [6].

One approach to obtaining information about functionally important amino acids and regions within polypeptides is to study the behaviour of hybrid enzyme complexes. Therefore, we have started to look into the possible formation of functional F_0 moieties derived from subunits of spinach chloroplasts and *E. coli*. As a first attempt, we have chosen the proteolipid subunit, also known as the DCCD-binding protein, which is thought to play an essential role in proton translocation.

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Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; SDS, sodium dodecyl sulfate; IPTG, isopropyl β-D-thiogalactopyranoside

2. MATERIALS AND METHODS

2.1. *Enzymes and chemicals*

Enzymes and agarose used for recombinant DNA techniques were obtained from Pharmacia (Freiburg), Boehringer-Mannheim and Gibco/BRL (Eggenstein). Immunochemicals and antibiotics were purchased from Sigma (Deisenhofen), nitrocellulose membranes (0.45 μm) from Schleicher & Schüll (Dassel), and protein standard from BioRad (München). Other chemicals were of analytical grade.

2.2. *Bacterial strains and plasmids*

Experiments were carried out using strain MJM413 which contains a Leu-4 → amber substitution in the *uncE* gene (*recA, srl*::Tn*10, thi,* Δ *uncE*334). This strain was kindly provided by Dr R.H. Fillingame (Madison). Plasmid pWHsp306/E2 was a gift from Dr R.G. Herrmann (Munich), pJLA504 from Dr J.E.G. McCarthy (Braunschweig). Expression vector pTTQ19 was obtained from Amersham Buchler (Braunschweig). Plasmids pBR322 and pUC18 were purchased from Pharmacia (Freiburg).

2.3. *Recombinant DNA techniques*

Standard procedures were used as described previously [7,8]. DNA fragments were recovered from agarose gels by the 'freeze-squeeze' method [9] except that a phenol extraction was added before the ethanol precipitation step. Transformation of *E. coli* cells was carried out as outlined by Chung et al. [10].

2.4. *Media and growth of cells*

For analytical procedures cells were grown in LB medium [7]. Complementation studies were carried out on minimal medium agar plates [11] containing either 0.4% glucose or succinate as carbon source. All media were supplemented with 2 μg/ml thiamin and 50 μg/ml of the appropriate antibiotics (final concentrations). Gene expression under control of the *tac* promoter was induced by 0.2 mM IPTG (final concentration).

2.5. *Analytical procedures*

SDS-gel electrophoresis was carried out according to Schägger and von Jagow [12] using gels with 16% acrylamide and 6 M urea. Western blot procedures were as published by Hensel et al. [13]. Membrane separation was performed as described by Osborn and Munson [14].

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3. RESULTS

3.1. *Plasmid construction*

In order to achieve an expression of subunit *III* in *E. coli* the *atpH* gene was cloned into different vectors. In previous attempts with both plasmid pBR322 and pUC18, no expression could be observed, whereas with the heat-inducible expression vector pJLA504 [15] expression could be shown. Unfortunately, the synthesized proteolipid was degraded within 20–25 min after heat induction (data not shown). Since we suspected that this could be due to the co-induction of a heat-shock protease, we cloned a *NsiI/BamHI* fragment of pWHsp306/E2, carrying the *atpH* gene and an upstream region containing a possible '– 10' and '– 35' consensus sequence and the putative ribosome binding site [6], into the *PstI/BamHI* sites of the IPTG-inducible expression vector pTTQ19 (Fig. 1).

3.2. *Analysis of gene expression*

Since in SDS-gels the proteolipid co-migrates with a broad band of lipids, making staining very difficult, the expression of subunit *III* in *E. coli* cells (Fig. 2) was demonstrated by Western blot analyses, using antibodies against the spinach chloroplast proteolipid raised in rabbits [16].

The location of the synthesized proteolipid was determined by membrane separation. It has been found that the protein is inserted predominantly into the bacterial cytoplasmic membrane (Fig. 3). A smaller amount of the proteolipid has also been found in the outer membrane fraction, but this is probably a preparation artefact.

3.3. *Complementation studies*

As an assay for functional ATP synthase complex formation, growth of the *uncE* mutant strain MJM413 on succinate, a non-fermentable carbon source, was tested. Complementation was, however, not observed.

4. DISCUSSION

Expression of subunit *III* of the ATP synthase from spinach chloroplasts in *E. coli* has been successfully

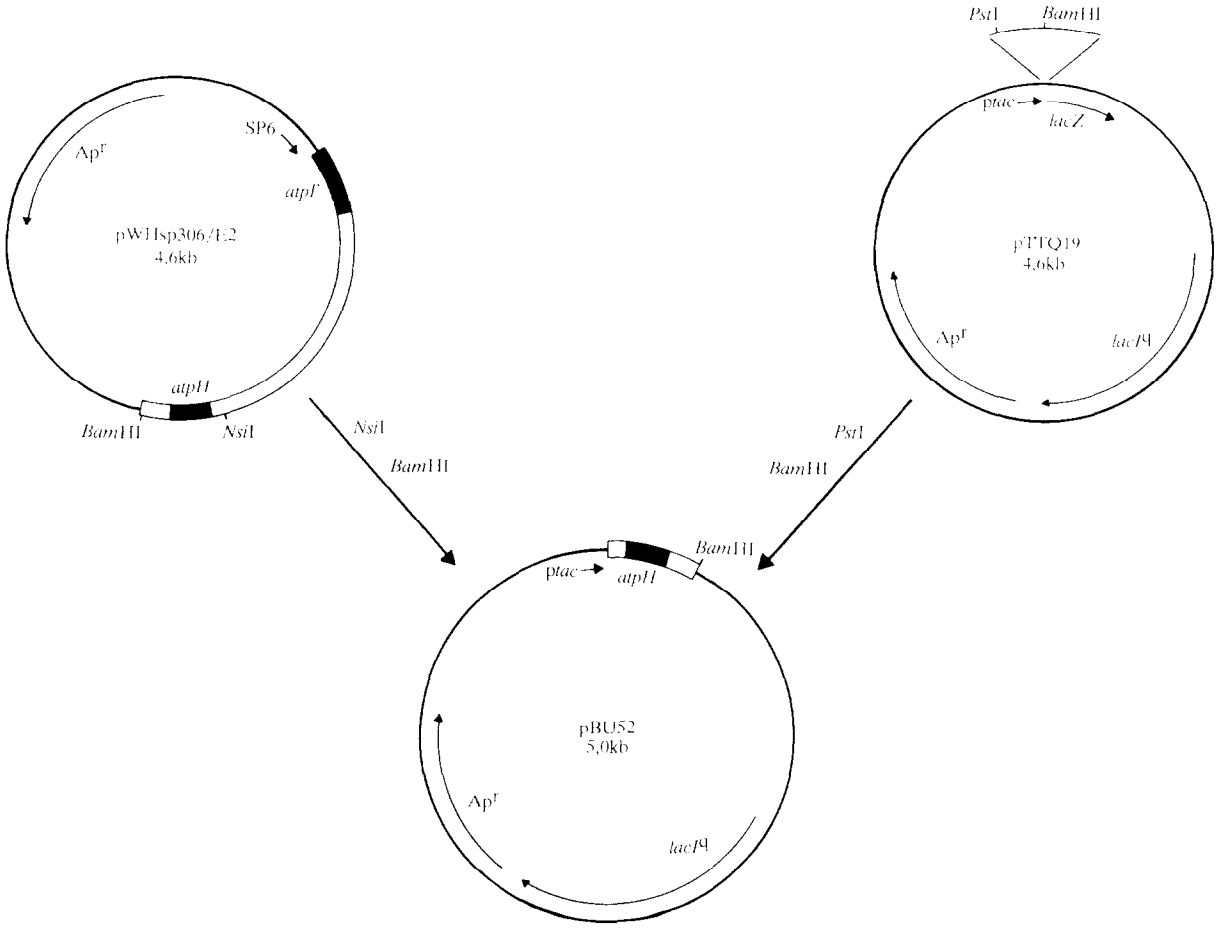

Fig. 1. Construction of plasmid pBU52.

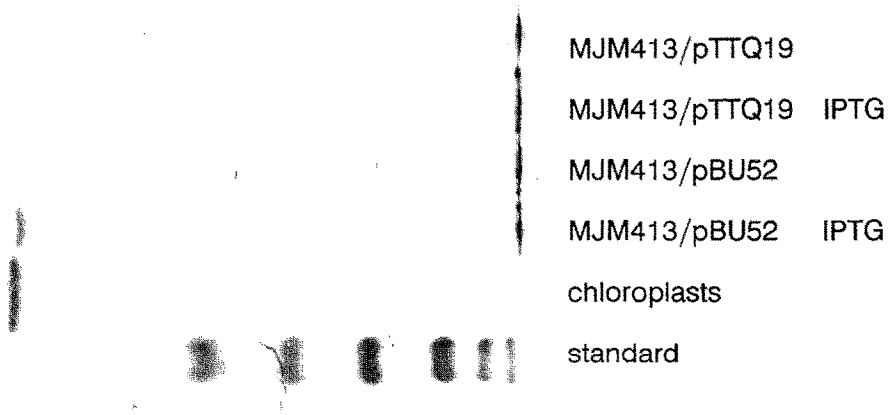

Fig. 2. Expression of CF_0 subunit *III* in intact cells of *E. coli*. Identical amounts of cells (20 μg) from strain MJM413 carrying either plasmid pTTQ19 or pBU52 were loaded onto the gel. After SDS-gel electrophoresis the proteins were blotted onto nitrocellulose membranes and the proteolipid was positively identified by antibodies. As a control a chloroplast preparation (10 μg) was used. The standard contains proteins with the molecular weights of 110 000, 84 000, 47 000, 33 000, 24 000, and 16 000.

achieved. Although the protein is predominantly inserted into the bacterial cytoplasmic membrane, formation of a functional F_0 complex was not observed. In contrast, as recently reported [17], CF_0 *I* is able to complement an *uncF* mutation in *E. coli*. This observation poses the question why CF_0 *III* cannot replace EF_0 *c* although the predicted secondary structures are quite similar [18]. One possibility could be that in contrast to all other known proteolipids the DCCD-binding residue in *E. coli* is an aspartic acid whereas in chloroplasts there is a glutamic acid at position 61 of the polypeptide chain. Unpublished results by Dr R.H. Fillingame (Madison) had shown that Asp-61 in EF_0 *c* is essential for proton translocation because a glutamic acid residue at the same position leads to a non-functional F_0 complex. Therefore, we would like to replace the CF_0 *III* Glu-61 by aspartic acid, using site-directed mutagenesis. The other possibility that certain regions within the proteolipid are also essential for proper function will be tested by the in vivo formation of hybrid proteolipids comprising regions of both EF_0 *c* and CF_0 *III* [19,20].

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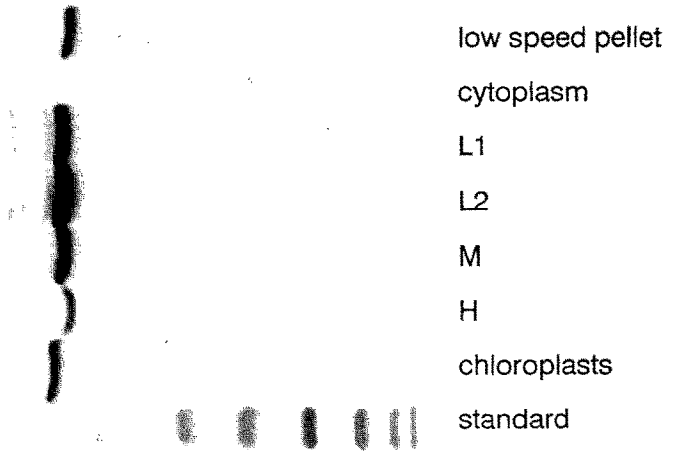

Fig. 3. Membrane separation of strain MJM413 carrying plasmid pBU52. IPTG-induced cells were subjected to the membrane separation procedure described in Materials and Methods. The fractions obtained by sucrose gradient centrifugation were washed once and subjected together with the low-speed pellet and the cytoplasm to SDS-gel electrophoresis (10 μg of each). Fractions L1 and L2 represent the cytoplasmic membrane, fraction M contains both, cytoplasmic and outer membrane vesicles, and the outer membrane is concentrated in fraction H. Western blot analysis, control and standard proteins are as described as in the legend of Fig. 2.

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